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Polymorphisms in ATP-binding cassette transporters associated with maternal methylmercury disposition and infant neurodevelopment in mother-infant pairs in the Seychelles Child Development Study

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ABSTRACT

Background: ATP-binding cassette (ABC) transporters have been associated with methylmercury (MeHg) toxicity in experimental animal models.
Aims: To evaluate the association of single nucleotide polymorphisms (SNPs) in maternal ABC transporter genes with 1) maternal hair MeHg concentrations during pregnancy and 2) child neurodevelopmental outcomes.
Materials and methods: Nutrition Cohort 2 (NC2) is an observational mother-child cohort recruited in the Republic of Seychelles from 2008–2011. Total mercury (Hg) was measured in maternal hair growing during pregnancy as a biomarker for prenatal MeHg exposure (N = 1313) (mean 3.9 ppm). Infants completed developmental assessments by Bayley Scales of Infant Development II (BSID-II) at 20 months of age (N = 1331). Genotyping for fifteen SNPs in *ABCC1*, *ABCC2* and *ABCB1* was performed for the mothers.
Results: Seven of fifteen ABC SNPs (*ABCC1* rs11075290, rs212093, and rs215088; *ABCC2* rs717620; *ABCB1* rs10276499, rs1202169, and rs2032582) were associated with concentrations of maternal hair Hg ($p < 0.001$ to 0.013). One SNP (*ABCC1* rs11075290) was also significantly associated with neurodevelopment; children born to mothers with rs11075290 CC genotype (mean hair Hg 3.6 ppm) scored on average 2 points lower on the Mental Development Index (MDI) and 3 points lower on the Psychomotor Development Index (PDI) than children born to mothers with TT genotype (mean hair Hg 4.7 ppm) while children with the CT genotype (mean hair Hg 4.0 ppm) had intermediate BSID scores.
Discussion: Genetic variation in ABC transporter genes was associated with maternal hair Hg concentrations. The implications for MeHg dose in the developing child and neurodevelopmental outcomes need to be further investigated.

1. Introduction

Fish is a primary source of protein for more than four billion people worldwide (FAO/WHO, 2011). Methylmercury (MeHg) is a well-documented neurotoxicant that is present in all fish to a varying extent. At adequate dosages, MeHg poses a risk for the developing fetus, as it is known to readily cross the placenta and the blood-brain barrier (WHO, 2007). However, the results concerning consequences of methylmercury exposure naturally found in fish on child neurodevelopment has been contradictory. While studies in

Abbreviations: ABC, ATP-binding cassette; ABCB1, ATP-Binding Cassette Sub-Family B (MDR/TAP) Member 1; ABCC1, ATP-Binding Cassette Sub-Family C (CFTR/MRP) Member 1; ABCC2, ATP-Binding Cassette Sub-Family C (CFTR/MRP) Member 2; BSID-II, Bayley scales of infant development II; MDI, Mental Development Index; MDR1, multidrug resistance protein 1; MeHg, methylmercury; MRP1, Multidrug resistance-associated protein 1; MRP2, Multidrug resistance-associated protein 2; NC2, Nutrition Cohort 2; PDI, Psychomotor Development Index; SCDS, Seychelles Child Development Study; SNP, single nucleotide polymorphism.

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New Zealand, Faroe Islands, and the United States have reported adverse developmental influences associated with prenatal MeHg exposure (Crump et al., 1998; Grandjean et al., 1997; Oken et al., 2005; Sagiv et al., 2012), no overall association between prenatal MeHg exposure and developmental outcomes were observed in large studies in the Seychelles, United Kingdom and Spain (Daniels et al., 2004, Davidson et al., 1998; Llop et al., 2012; Myers et al., 2003; Strain et al., 2015). These results reveal that the association between prenatal MeHg exposure from maternal fish consumption and child developmental outcomes is far more complex than previously thought and inconsistencies among study findings may be due to variability in concomitant dietary exposures or genetic factors influencing MeHg toxicity. Several factors influence the concentration of MeHg experienced by the fetus, such as the type and the amount of fish consumed; however, the contribution of the genetic background has rarely been studied. Sequence variation in maternal genes responsible for the toxicokinetics of MeHg is likely to result in differences in MeHg concentrations, and in turn differences in neurotoxicity, between infants of mothers with a similar fish intake. Yet, very little is known about gene – MeHg interactions on child neurodevelopment (Llop et al., 2015).

Animal studies provide evidence that transporter proteins can affect MeHg toxicokinetics. The ATP-binding cassette (ABC) transporters Multidrug resistance-associated protein 1 (MRP1, gene name ATP-Binding Cassette, Sub-Family C (CFTR/MRP), Member 1 (*ABCC1*)), Multidrug resistance-associated protein 1 (MRP2, gene name ATP-Binding Cassette, Sub-Family C (CFTR/MRP), Member 2 (*ABCC2*)) and multidrug resistance protein 1 (MDR1, gene name ATP-Binding Cassette, Sub-Family B (MDR/TAP), Member MDR1 (*ABCB1*)) are the best-characterized ABC transporters. *ABCC1* and *ABCC2* have been associated with MeHg accumulation and toxicity in fruit flies and mice (Bridges and Zalups, 2005; Prince et al., 2014; Toyama et al., 2011). The role of *ABCB1* in MeHg transport and toxicity is not yet known. These three ABC transporter genes are highly conserved across species and are abundant in tissues where MeHg transport is most critical (*e.g.* blood-brain-barrier, liver, gut and the placenta). The role of ABC transporters in MeHg transport suggests that single nucleotide polymorphisms (SNPs) in ABC transporters may influence MeHg body burden in both mother and fetus during pregnancy. Indeed, in a study based on two European birth cohorts, it was found that child SNPs in *ABCC1*, *ABCC2*, and *ABCB1* modified the relationship between maternal fish consumption and cord blood Hg concentrations (Llop et al., 2014). However, it is not known whether ABC SNPs influence the concentration of maternal hair Hg, a biomarker often used as a proxy for prenatal exposure to MeHg (Davidson et al., 2008). The potential influence of ABC genotype on MeHg body burden in pregnant mothers or their children may mediate adverse associations with developmental outcomes; however, few studies have investigated the association between ABC SNPs and child neurodevelopment directly. The current study comprises a mother-child cohort from the Seychelles Child Development Study (SCDS), characterized by high fish intake and hair Hg concentrations which are substantially higher than in many other populations. Here, we characterize the associations of maternal SNPs in ABC transporters with hair Hg concentrations during pregnancy and with child neurodevelopmental outcomes.

2. Methods

2.1. Study population

The SCDS is a longitudinal observational study conducted in the Republic of Seychelles, an archipelago in the Indian Ocean. The population resides mainly on the island of Mahé and is of mixed African, European, and East Asian origin. The overall aim of the SCDS is to investigate the effects of MeHg exposure during pregnancy on child developmental outcomes. Healthy mothers were recruited to Nutrition Cohort 2 (NC2) during their first antenatal visit (from 14 weeks of gestation) at eight health centres across Mahé. Mothers were enrolled from 2008 until 2011 when the target number of 1500 mothers had consented (Strain et al., 2015). Mothers with double pregnancies were only counted once in analyses of developmental outcomes (one of each sibling pair was removed randomly). One hair Hg value was an outlier (194.3 ppm, the next highest observed was 31.66 ppm); the Hg was found to be primarily inorganic and this value was therefore excluded from the Hg analyses. After exclusions owing to missing data (Strain et al., 2015), there were 1313 mothers who were included in the analyses of MeHg toxicokinetics and 1331 mother-child pairs were included in analyses of developmental outcomes. Further information on inclusion criteria and power calculations has previously been described (Strain et al., 2015). The study was reviewed and approved by the Seychelles Ethics Board, the Research Subjects Review Board at the University of Rochester, and the Regional Ethics Committee at Lund University, Sweden.

2.2. Hair Hg analyses

Hair samples were cut at delivery and the longest available segment of maternal hair growing during gestation was analyzed assuming a hair growth rate of 1.1 cm/month. Total mercury in maternal hair during gestation is a known biomarker for prenatal MeHg exposure and was measured by cold-vapor Atomic-Absorption-Spectrometry as previously described (Cernichiari et al., 1995) and reported in parts per million (ppm).

2.3. Developmental assessment

When infants were aged approximately 20 months, they completed developmental testing with a Creole version of the Bayley Scales of Infant Development (BSID-II), which has been successfully used in a previous Seychellois cohort (Davidson et al., 2008) as well as in the current NC2 cohort (Strain et al., 2015). Testing was conducted by specially trained nurses at the Child Development Centre, Victoria, Mahé. All study forms were shipped to the University of Rochester, where data were double-entered and the Mental Development Index (MDI) and Psychomotor Development Index (PDI) endpoints were scaled according to the child's age at testing. Test reliability for the BSID-II was determined as previously described (Strain et al., 2015).

2.4. DNA extraction and genotyping

DNA was extracted from maternal blood using the Qiagen DNA Blood Mini kit (Qiagen, Hilden, Germany). SNPs were genotyped by the iPLEX® Gold assay on the MassARRAY platform (SequenomTM, San Diego, USA) and by TaqMan allelic discrimination assay on an ABI 7900 instrument (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. We characterized the variation in the ABC genes by choosing TagSNPs that capture as much of the genetic variation within a gene as possible due to linkage disequilibrium with other (not genotyped) SNPs. TagSNPs were selected according to Hapmap data (Thorisson et al., 2005) for YRI (Yoruba in Ibadan, Nigeria) as a proxy for African populations, since earlier screenings of this population have shown that the allele frequencies are more similar to YRI than the other Hapmap populations (Yeates et al., 2015). In total, we genotyped 15 SNPs (description of all SNPs is found in Table 1) including the three SNPs that previously had been linked to cord blood Hg in Mediterranean cohorts: *ABCC1* rs11075290, *ABCC2* rs2273697, and *ABCB1* rs2032582 (Llop et al., 2014). *ABCB1* rs2032582 is tri-allelic and was genotyped by two different TaqMan assays to capture all three alleles. Five percent of the samples were re-analyzed for quality control purposes with perfect agreement between original and repeat genotyping runs for all SNPs.

Table 1
Single nucleotide polymorphisms (SNPs) included in the NC2 study. All genotyped individuals included in either the association analyses with hair Hg or with BSID analyses are included (n = 1410–1416 depending on the SNP).

Gene	SNP[a]	SNP type[b]	Functional effect[c]	Allele freq. NC2	Allele freq. YRI[d]	Allele freq. CEU[e]
ABCC1	rs215088	Intronic A/G	Regulatory variant - enhancer	0.41 (A)	0.59	0.29
ABCC1	rs12927980	Intronic G/T	Regulatory variant - promotor flanking region	0.05 (T)	0.10	0.08
ABCC1	rs11075290	Intronic C/T	Regulatory variant - promotor flanking region	0.37 (T)	0.30	0.55
ABCC1	rs246241	Intronic G/T	Regulatory variant - promotor flanking region	0.24 (T)	0.26	0.11
ABCC1	rs212093	5′ near gene G/A	No predicted change of function	0.41 (A)	0.38	0.57
ABCC2	rs717620	5′ untranslated region C/T	Regulatory variant – promotor	0.10 (T)	0.03	0.21
ABCC2	rs2756103	Intronic A/C	Regulatory variant - promotor flanking region	0.29 (C)	0.23	0.42
ABCC2	rs7393105	Intronic A/C	No predicted change of function	0.36 (C)	0.40	0.42
ABCC2	rs2273697	Non-synonymous, Val(G)/Ile(A)	Predicted tolerated	0.20 (A)	0.25	0.20
ABCB1	rs2032582	Non-synonymous, Ala(G)/Ser(T)/Thr(A)	Ser → Ala predicted tolerated Ser → Thr predicted deleterious	0.79(G)/0.18(T)/0.02(A)	100 (G)	0.56/0.43/0.01
ABCB1	rs2235035	Intronic G/A	No predicted change of function	0.21 (A)	0.21	0.31
ABCB1	rs10274587	Intronic G/A	No predicted change of function	0.16 (A)	0.20	0.13
ABCB1	rs1202169	Intronic T/C	No predicted change of function	0.34 (C)	0.19	0.42
ABCB1	rs1202171	Intronic T/A	No predicted change of function	0.25 (T)	0.36	0.37
ABCB1	rs10276499	Intronic C/T	No predicted change of function	0.38 (C)	0.35	0.06

[a] SNPs are ordered according to their position in the gene at 5′ to 3′ direction, according to Human genome assembly 38 (GRCh38). *ABCB1* is situated on chromosome 7, *ABCC1* on chromosome 16, and *ABCC2* on chromosome 10.
[b] Ancestral allele is denoted first.
[c] According to www.ensembl.org, amino acid exchange predictions are from "Sorting Intolerant from tolerant" (SIFT) and from PolyPhen-2 (Polymorphism Phenotyping v2) at Ensembl.
[d] Data from Hapmap (www.hapmap.org) for an African population (YRI, Yoruba in Ibadan, Nigeria).
[e] Data from Hapmap (www.hapmap.org) for a European population (CEU, Utah Residents with Northern and Western European ancestry).

2.5. Statistical analyses

Deviations from Hardy-Weinberg equilibrium were tested using chi-square analysis. Linkage disequilibrium was evaluated using Haploview (Barrett et al., 2005). Tests for associations between outcomes and SNPs were carried out from *a priori* analysis plans and all associations were evaluated using two-sided tests of significance at the $\alpha = 0.05$ level. For the tri-allelic *ABCB1* rs2032582 we excluded genotypes with the A-allele due to allele frequency < 5% (there were 60 subjects with GA, AT and AA genotype removed from the analysis of genetic association with maternal hair Hg concentrations and 61 from the analysis of genetic associations with child BSID-II); after this removal there were three SNP levels. All statistical analyses were undertaken using R (version 3.0.2; The R Foundation for Statistical Computing).

Simple linear regression with no covariate adjustment (assuming that fish consumption patterns and other determinants of MeHg exposure are similar for mothers with different SNPs) was used to estimate the association of each of the ABC SNPs with maternal hair Hg concentrations. We used a 2 degree of freedom test to evaluate differences in hair Hg across the three levels of each SNP. Multiple linear regression was also used to estimate the association of ABC SNPs with BSID-II scores and these were adjusted for covariates previously chosen to cover the most important determinants of neurocognitive development in children (Strain et al., 2015).

The covariates included child sex, maternal age at delivery, presence of two parents in the household, Hollingshead socioeconomic score, and child age at testing. The models for the BSID MDI and PDI did not include maternal hair Hg because as a potential mediator it would affect our ability to estimate the direct association between SNPs and BSID scores. Because there were many missing values for maternal hair Hg, our sample sizes for these analyses were considerably larger than those reported in Strain et al. (2015).

3. Results

Summary statistics for the cohort are shown in Table 2. All SNPs were in Hardy-Weinberg equilibrium. *ABCC2* (rs7393105 and rs2756103) were in linkage disequilibrium ($R^2 = 0.72$), while all other pairwise combinations of SNPs had a R^2 lower than 0.3.

SNPs in all three ABC genes were significantly associated ($p < 0.050$) with maternal hair Hg (Table 3). None of these SNPs were in linkage disequilibrium. Three SNPs in *ABCC1* (rs11075290, rs212093, and rs215088), one SNP in *ABCC2* (rs717620), and three SNPs in *ABCB1* (rs10276499, rs1202169, and rs2032582) were associated with hair Hg concentrations, and all but two (*ABCC1* rs212093 and rs215088) showed an allele-concentration effect. For example, the mean hair Hg among CC carriers of rs11075290 was 1 ppm (~20%) lower than the mean hair Hg among TT carriers. Using Hapmap data, we compared this population with those of African and European ancestry. The alleles associated with higher concentrations of Hg in hair in this study (Table 3) showed in general a higher allele frequency in Hapmap individuals with European ancestry, but a lower allele frequency in Hapmap individuals with African ancestry, compared with in NC2 (Table 1).

In our analysis of maternal ABC genotypes in relation to the neurodevelopment of their children we found that only *ABCC1* rs11075290 was significantly associated with MDI and PDI (Table 3). Mothers with the CC variant had the lowest hair Hg concentrations, but the children born to them showed poorer performance. Their children averaged 2 points lower for the MDI and 3 points lower for the PDI than children born to mothers with TT variant who had higher hair Hg. No other clear patterns of associations were found between the BSID-II outcomes and ABC SNPs.

4. Discussion

This study suggests that genetic differences in ABC transporters influence human MeHg body burden; seven out of 15 ABC SNPs genotyped were significantly associated with concentrations of Hg in hair

Table 2
Summary statistics for the Nutrition Cohort 2 in the Seychelles Child Development Study.

Variable	N	Mean	SD
Maternal hair Hg (ppm)	1313	3.94	3.47
Fish meals per week	1243	8.58	4.63
Birthweight (g)	1305	3172	498
Gestational age (weeks)	1262	38.96	1.61
BSID score MDI	1327	87.63	10.74
BSID score PDI	1325	96.69	10.59
% Girls	1331	48%	
% Two parents in the household	1331	73%	
Maternal age (years)	1331	27.06	6.29
Hollingshead SES	1331	32.04	10.35

Abbreviations: N = number of individuals, SD = standard deviation, Hg = mercury.

Table 3
Associations between maternal *ABC* genotypes, maternal hair Hg, and the BSID MDI and PDI[a].

Gene/SNP		Hair Hg			MDI				PDI			
		N	Mean	p	N	Mean	ß	p	N	Mean	ß	p
ABCC1 (*MRP1*)												
rs215088	GG	470	4.3	**0.013**	480	87.6	–	0.34	479	96.8	–	0.42
	GA	622	3.7		621	88.0	0.42		621	96.3	−0.39	
	AA	221	3.8		226	86.8	–		225	97.4	0.67	
rs12927980	GG	1184	3.9	0.09	1197	87.6	–	0.70	1196	96.5	–	0.080
	GT	125	4.4		127	88.4	0.85		126	98.7	2.47	
	TT	4	6.5		3	87.3	0.27		3	95.7	−1.26	
rs11075290	CC	543	3.6	<**0.001**	545	87.2	–	**0.042**	547	95.7	–	**0.002**
	CT	553	4.0		560	87.4	0.27		556	97.0	1.23	
	TT	211	4.7		216	89.2	1.92		216	98.6	2.95	
rs246241	GG	746	4.0	0.22	756	87.8	–	0.52	754	96.9	–	0.60
	GT	492	4.0		493	87.5	−0.17		493	96.3	−0.52	
	TT	75	3.3		78	86.4	−1.09		78	97.1	0.38	
rs212093	GG	460	3.6	**0.007**	468	88.2	–	0.36	467	96.7	–	0.78
	GA	638	4.2		638	87.3	−0.94		638	96.9	0.14	
	AA	213	3.8		219	87.5	−0.85		218	96.3	−0.45	
ABCC2 (*MRP2*)												
rs717620	CC	1056	3.8	<**0.001**	1066	87.8	–	0.52	1065	96.8	–	0.41
	CT	244	4.4		249	87.2	−0.23		248	96.0	−0.72	
	TT	13	7.6		12	85.1	−2.09		12	98.7	1.92	
rs2756103	AA	679	3.8	0.25	689	87.8	–	0.63	685	96.9	–	0.60
	AC	523	4.0		528	87.5	−0.34		529	96.3	−0.51	
	CC	111	4.4		110	86.9	−0.87		111	97.0	0.07	
rs7393105	AA	545	3.9	0.52	554	87.8	–	0.88	551	97.0	–	0.47
	AC	605	3.9		608	87.5	−0.28		608	96.3	−0.73	
	CC	163	4.2		165	87.4	−0.22		166	96.9	−0.11	
rs2273697	GG	843	3.9	0.94	849	87.8	–	0.64	849	96.6	–	0.89
	GA	421	3.9		428	87.3	−0.31		427	96.9	0.37	
	AA	49	4.1		50	87.3	−0.52		49	96.2	−0.45	
ABCB1 (*MDR1*)												
rs2032582	GG	840	3.6	<**0.001**	848	87.5	–	0.077	848	96.9	–	0.11
	GT	357	4.4		364	87.7	0.09		364	96.1	−0.86	
	TT	56	5.6		54	90.8	1.73		54	99.1	1.75	
rs2235035	GG	821	3.9	0.69	832	87.7	–	0.96	832	96.3	–	0.30
	GA	430	4.0		434	87.5	−0.15		434	97.3	0.88	
	AA	62	4.3		61	87.7	0.02		59	97.4	0.94	
rs10274587	GG	929	4.0	0.58	942	87.5	–	0.54	939	96.8	–	0.74
	GA	341	3.8		340	87.6	0.07		341	96.3	−0.56	
	AA	42	3.6		44	89.3	1.85		44	97.2	0.45	
rs1202169	TT	564	3.7	**0.006**	572	87.5	–	0.92	570	97.2	–	0.32
	TC	599	4.0		606	87.7	−0.11		606	96.3	−0.95	
	CC	148	4.7		147	87.9	−0.18		147	96.4	−0.87	
rs1202171	AA	745	4.1	0.25	753	87.6	–	0.63	749	96.7	–	0.99
	AT	499	3.7		498	87.4	−0.17		500	96.7	−0.05	
	TT	69	4.0		76	88.7	1.03		76	96.6	0.09	
rs10276499	TT	507	4.4	<**0.001**	510	87.9	–	0.065	509	96.3	–	0.27
	TC	602	3.8		610	87.9	0.23		609	97.2	0.86	
	CC	203	3.2		206	86.1	−1.37		206	96.1	−0.13	

[a] The hair Hg model is unadjusted, so the p-values test for differences between the three means. The homozygote for the most common allele is used as reference. The beta coefficients (β) for the MDI and PDI models are adjusted for child sex, maternal age at delivery, presence of two parents in the household, Hollingshead socioeconomic score, and child age at testing. The p-values from the sequential sum of squares test for differences in the effect of SNP and are unadjusted for the other covariates. SNP: single nucleotide polymorphism. Bold values indicates all associations were evaluated using two-sided tests of significance at the $\alpha=0.05$ level.

among mothers in a cohort with high fish consumption and a mean hair Hg concentration of 3.9 ppm. Among the various maternal ABC transporter genotypes that were associated with Hg in hair, only one SNP, in *ABCC1* (rs11075290), was associated with child BSID scores. No other clear pattern of association with developmental outcomes was observed. Based on the strong association of ABC genotype with maternal hair Hg concentrations we predict that the ABC transporter genotype of the child may also play a role in the child's MeHg body burden. Importantly, these transporters are intrinsic components of the placenta and the blood-brain barrier and thus could influence brain exposure to MeHg in the developing child.

It should however be stressed that ABC transporters transports a number of different molecules and are thus not specific for MeHg transport. Consequently, the associations between ABC transporters and neurodevelopment may be blurred by the transport of other compounds that may influence neurodevelopmental outcomes. The involvement of ABCC1/MRP1 in human MeHg distribution and elimination was suggested by findings from experimental studies. Knocking down *ABCC1* in fruit flies resulted in accumulation of MeHg and greater developmental toxicity measured as eclosion (Prince et al., 2014) and over-expression of the ABCC1/MRP1 protein in mice resulted in decreased accumulation of MeHg in brain and liver (Toyama et al., 2011). However, we do not presently know the role of ABCC1/MRP1 in altering MeHg body burden in humans, and particularly during pregnancy. The ABCC1/MRP1 protein is expressed in several tissues that are critical in absorption, distribution and excretion of MeHg, both in the mother and the fetus. ABCC1/MRP1 expression occurs in many tissues (www.proteinatlas.org), including the placenta (Aye et al., 2007), the blood-brain barrier (Leslie et al., 2005), the intestine (Berggren et al., 2007) and the hair follicle (Haslam et al., 2013). Its expression in the

hair follicle is of particular relevance when considering hair as the biomarker of exposure as used in this study. During hair synthesis MeHg from the bloodstream is transported to keratinocytes in the follicle that, subsequent to differentiation and death, are incorporated in the matrix of the growing hair shaft (Kempson and Lombi, 2011; Zareba et al., 2008). Nonetheless, information about the apical/basal orientation of ABCC1/MRP1 within the hair follicle, and other tissues, is limited and it is therefore difficult to predict how the genotype ultimately influences the kinetics of MeHg transport and distribution in various compartments of the body.

Among the 15 SNPs investigated, just one SNP, rs11075290 in *ABCC1*, was associated with both maternal hair Hg concentrations and child BSID scores. It is positioned in the first intron of the *ABCC1* gene in a promoter flanking region. The C allele creates a CpG site, which can be methylated and potentially result in lower expression of *ABCC1*. Nevertheless, the function of this SNP has not been tested *in vitro* and is uncertain. Its impact on MeHg transport and distribution at the level of the placental/fetal circulation remains unclear. Thus, in contrast to the strong association between maternal ABC genotype and a maternal mercury disposition phenotype, the impact at the level of developmental phenotype in the offspring is less certain. The association with developmental phenotype is likely to be determined also by the child's ABC genotype. Future analyses incorporating the children's ABC SNPs are needed to determine if there are susceptible sub-populations that can be identified. It is also possible that our findings reflect linkage disequilibrium of this SNP with other functional SNPs not yet characterized.

We did not include Hg in hair in the model for the association between ABC genotype and neurodevelopment. If ABC genotype would have been clearly associated with neurodevelopmental outcomes then it would have been appropriate to conduct a mediation model including Hg in hair. However, in the absence of clear associations of ABC genotype with neurodevelopmental outcomes as well as Hg in hair and neurodevelopmental outcomes (Davidson et al., 1998; Myers et al., 2003; Strain et al., 2015), and recognizing the broad functions of ABC transporters, we did not conduct a mediation analysis that included Hg in hair as a covariate in the model.

We found that additional SNPs in *ABCC1*, *ABCC2*, and *ABCB1* were also associated with hair Hg, and are thus candidates to explore for a role in MeHg transport, distribution and elimination. The ABC SNPs varied in allele frequency when comparing NC2 with individuals from Hapmap with European and African ancestry: alleles associated with elevated Hg in hair were in general higher in frequency in Hapmap individuals with European ancestry. Further studies on the role of ABC SNPs for MeHg concentrations in different tissues, such as blood and brain, will facilitate understanding of individual differences in Hg distribution and elimination. Such studies will potentially help us to identify susceptible groups. An earlier study found that the promoter-associated rs717620 (http://www.ensembl.org) was correlated with urinary Hg concentrations (a marker of inorganic Hg) among gold miners (Engstrom et al., 2013). The rs717620 A-allele carriers had higher Hg concentrations in urine than individuals with the GG genotype. A parallel pattern was seen for hair Hg in this study, where the A-allele was associated with higher hair Hg concentrations, suggesting that this SNP may act to influence overall transport and elimination of mercury.

Strengths of this study include the large well-characterized cohort and extensive information about sequence variation in some genes encoding potential transporters for MeHg. The lack of genetic data for the children is a limitation, but further studies aim to characterize more fully the contribution of child genotype on MeHg body burden and cognitive outcomes.

5. Conclusions

We found that SNPs in genes coding for three ABC transporter genes are associated with maternal hair Hg concentrations in a population consuming large quantities of ocean fish. These findings suggest that genetics may play a role in determining maternal, and thus prenatal, MeHg exposure. While consistent associations with BSID outcomes were not observed, we cannot exclude the possibility that ABC transporter genes play a role in influencing child MeHg body burden and possibly neurodevelopmental outcomes. Further studies are needed to explore the functional consequences of these polymorphisms, importantly, extending the study to the child's ABC transporters, to fully understand effects on MeHg toxicokinetics and MeHg-related effects on neurodevelopment.

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